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Histochemical analysis of skeletal muscular tissues of pigs according to genotype *MYF 4*

(Short communication)

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Abstract

The results of histochemical analysis of 3 muscles – *m. triceps brachii* (MTB), *m. longissimus thoracitus* (MLT) and *m. rectus femoris* (MRF) – in 2 groups of pigs formed according to the genotypes *MYF 4* are presented in this study. Determination of *MYF 4* genotypes was performed by the polymerase chain reaction method (PCR). For histochemical analysis, 5 animals detected as homozygotes of the *MYF 4-AA* type and 5 animals of the heterozygous genotype AB were used out of the total 25 individual animals tested. The histochemical analysis demonstrated that homozygotes AA had larger fat cells on the average than heterozygotes AB in the three studied muscles, which was statistically significant ($P<0.05$). The percentage surface representation of interstitial tissues was higher in the studied muscles of heterozygotes *MYF 4-AB*. The volume of interstitial tissues was the highest in the MRF in both groups (myogenin – AA and AB). The average thickness of muscle fibres of the three studied muscles was higher in the homozygote genotype myogenin *MYF 4-AA* than in heterozygotes *MYF 4*-AB, which was statistically significant ($P<0.05$).

Keywords: pigs, histology, MYF-4, myogenin, skeletal muscles

Zusammenfassung

Histochemische Analyse der Schweinemuskulatur in Verbindung mit dem Genotyp *MYF 4* (Kurzmitteilung)

Es erfolgten bei 25 Landrasse Mastschweinen mit einem Durchschnittsgewicht von 96,3 kg (±3 kg), welche nach der mit Hilfe der Polymerase-Kettenreaktion Methode (PCR) bestimmten MYF Genotypen zwei Gruppen zugeordnet wurden, histochemische Untersuchungen an den Muskeln *m. triceps brachii* (MTB), *m. longissimus thoracitus* (MLT) und *m. rectus femoris* (MRF). Für die histochemischen Analysen wurden 5 homozygote Schweine des Genotyps *MYF 4*-AA und 5 des Genotyps AB ausgewählt. Die homozygoten Genotypen wiesen bei allen drei untersuchten Muskeln größere Fettzellen auf. Die Fettzellengröße im MLT war gegenüber den anderen Muskeln in beiden Gruppen höher. Der Anteil des Interstitialgewebes war bei den heterozygoten Genotypen höher. Der höchste Anteil dieses Gewebes wurde bei beiden *MYF 4* Genotypen (AA, AB) im MRF nachgewiesen. Die durchschnittliche Muskelfaserstärke war bei den homozygoten Myogeningenotypen *MYF 4*-AA im Vergleich zu den heterozygoten *MYF 4*-AB höher.

Schlüsselwörter: Schwein, Histologie, Myogenin, *MYF 4* Genotypen, Skelettmuskel

Introduction

Striated skeletal muscle is at present a subject of intensive research, as muscles represent one of the most important elements in human nutrition. Studies of muscle growth and meat quality have led to an increasing interest in the microscopic skeletal muscle structure of individual types of animals (KUCHENMEISTER and KUHN 2003, REHFELD *et al.* 2005, LE FAUCHEUR 2006). The relationship between qualitative and quantitative properties of skeletal muscles and the final meat product is also determined by the growth changes of individual farm animals (CHEREPANOV 2001). Histochemical methods allow us to evaluate the qualitative and quantitative differences in the final product. It is clear that there are definite quantitative differences among the individual farm animals which are under the genetic control of several genes (WIMMERS *et al.* 2005). One of these is myogenin (*MYF 4*), which is an essential activator of myogenesis (ERNST *et al.* 1994, HORAK *et al.* 2004). We can assume that their effect is correlated with the postnatal growth of skeletal muscles (MAKOVICKÝ 2005). There exists only a little information about the influences of the growth differentiation factor.

The objective of this study was to determine whether there exist some differences between *MYF 4* and the postnatal growth of skeletal muscles in pigs.

Material and methods

The Landrace breed of pigs was used for this experiment. They were reared under standard conditions (age, nutrition, stabling, etc). The average weight of the animals at the end of the experiment was 96.3 kg (±3 kg). Immediately after slaughter of the 25 animals we took blood samples in order to be able to detect the genotypes of the *MYF 4* gene. We also took samples of three muscle types – *m. triceps brachii* (MTB), *m. longissimus thoracicus* (MLT) and *m. rectus femoris* (MRF). Samples of size 1×1×1 cm were taken at the latest 30 min *post mortem*. They were then wrapped in aluminium foil Alobal and immediately frozen in liquid nitrogen. One sample was taken from each muscle of the pig.

Genetic analysis of the *MYF 4* gene was performed by the PCR technique, and on the basis of the detection of genotypes we performed histochemical analyses of 3 muscle types (MTB, MLT, MRF) from 5 homozygous animals (*MYF 4-AA*) and 5 heterozygous animals (*MYF 4-AB*).

Sections 10 µm thick were cut in a freezing microtome (type MTB) at a temperature of –19°C to –21°C and subsequently subjected to histological tests. The first series of samples was stained with translucent haematoxylin-eosin, and the second series was stained for succinic dehydrogenase (SDH) activity, in order to detect muscle fibre types. The third series of slices was stained with red oil »0« to detect the neutral lipids. The preparations were evaluated in a light-microscopic picture. The average thickness of muscular fibres, fat cells and the percentage content of interstitial tissues and muscular fibres were measured morphometrically in homozygotes MYF4-AA pigs and heterozygotes *MYF 4*-AB pigs. 10 places in each section were randomly chosen. A minimum of 500 fibres was evaluated from the samples. Objective evaluations were performed with an Olympus Provis light microscope with the software NIS-Elements. The results were evaluated by

statistical models. Distinctions among panels were tested using the SAS program with binate-test at the limit α=0.05.

Results and discussion

The analysis of fat tissues

We detected fat cells of various sizes and their localization in all three muscles studied in both genotypes (*MYF 4-AA* and *MYF 4-AB*). The real and comparative characteristics were achieved on the basis of morphometric measurement of fat cell size. The highest average size value of fat cells in homozygotes *MYF 4-AA* was in MLT – 41.10 µm. The size of fat cells in MTB – 39.70 µm was slighty smaller. The fat cells in MRF were considerably smaller, their diameter being 36.70 µm. The minimum and maximum values show that the variation in the size of fat cells in the studied muscles was very constant (Table 1).

Table 1
The size of fat cells in µm – homozygotes *MYF 4-AA*
Fettzellgröße in µm bei homozygoten MYF 4-AA

Trait	Mean	Minimum	Maximum	Dispersion	Standard deviation	Variation, %
MTB	39.70	26.00	54.07	54.51	7.38	18.60
MLT	41.10	24.00	56.00	64.39	8.02	19.52
MRF	36.70	24.00	50.00	49.11	7.01	19.09

The size of fat cells in MLT – 38.50 µm was again prevalent in the animals detected as heterozygotes AB. The smallest average thickness was in MTB – 34.85, µm and in MRF it was slightly higher – 35.70 µm. The diameter of fat cells in the three studied muscles in all these animals (AB) was smaller than in homozygotes (AA), but the variability was higher (Table 2). The results demonstrate that there is a statistically significant difference between the size of fat cell of homozygotes *MYF 4-AA* and the size of fat cells of heterozygotes *MYF 4-AB* ($P<0.05$). There is a statistically significant difference between average thickens MTB of homozygotes and MTB of heterozygotes ($P<0.05$). Differences between MLD of homozygotes and MLD of heterozygotes is statistically not significant ($P>0.05$). Differences between MRF of homozygotes and MRF of heterozygotes is statistically significant ($P<0.05$).

Table 2
The size of fat cells in µm – heterozygotes *MYF 4*-AB
Fettzellgröße bei heterozygoten MYF 4-AB

Trait	Mean	Minimum	Maximum	Dispersion	Standard deviation	Variation, %
MTB	34.85	20.00	54.00	86.18	9.28	24.64
MLT	38.50	20.00	54.00	85.15	9.23	23.97
MRF	35.70	24.00	52.00	50.71	7.12	19.95

The analysis of muscle fibres

Histological analysis showed that the average thickness of muscle fibres varies among the genetically investigated groups. But the thickness tendency in muscles is the same. The homozygotes *MYF 4-AA* showed higher average values of thickness of muscle fibres

in all three muscle types (MTB, MLT, MRF). Those of homozygotes AA had the highest average of muscular fibre thickness in MRF – 88.60 μm and the lowest in MTB – 73.30 μm. In MLT, muscle fibres had an average thickness of 79.03 μm. The heterozygotes *MYF 4-AB* had the same tendency of thickness in the direction of MRF (84.72 μm), MLT (75.77 μm) and MTB (69.40 μm) (Table 3 and 4). There is a statistically significant difference between average thickness of muscle fibre types of homozygotes *MYF 4-AA* and the average thickness of muscle fibre types heterozygotes *MYF 4-AB* ($P<0.05$). Differences between MTB of homozygotes and MTB of heterozygotes is statistically significant ($P<0.05$). Differences between MLD of homozygotes and MLD of heterozygotes is statistically significant ($P<0.01$). Differences between MRF of homozygotes and MRF of heterozygotes is statistically not significant ($P>0.05$).

Table 3
Average thickness of muscle fibre types – homozygotes *MYF 4-AA*
Muskelfaserdicke unterschiedlicher Muskelfasertypen bei MYF 4-*AA*

	Trait	Mean	Dispersion	Standard deviation	Variation, %
MTB	β-red	71.36	7.66	2.77	3.77
	α-red	73.24	7.61	2.76	3.77
	α-white	75.31	7.26	2.69	3.68
	total mean	73.30	7.51	2.74	3.74
MLT	β-red	79.32	8.38	2.89	3.65
	α-red	78.81	10.24	3.20	4.06
	α-white	78.95	9.42	3.07	3.89
	total mean	79.03	9.34	3.05	3.86
MRF	β-red	84.61	12.68	3.56	3.93
	α-red	84.61	12.15	11.02	13.03
	α-white	90.62	12.53	3.54	3.91
	total mean	88.60	12.45	6.04	6.95

Table 4
Average thickness of muscle fibre types – heterozygotes *MYF 4-AB*
Muskelfaserdicke unterschiedlicher Muskelfasertypen bei MYF 4-*AB*

	Trait	Mean	Dispersion	Standard deviation	Variation, %
MTB	β-red	66.40	2.90	1.70	2.45
	α-red	69.41	2.92	1.70	2.45
	α-white	72.40	2.91	1.71	2.46
	total mean	69.40	2.91	1.70	2.45
MLT	β-red	73.76	2.17	1.47	1.94
	α-red	75.78	2.14	1.46	1.93
	α-white	77.76	2.16	1.47	1.94
	total mean	75.77	2.15	1.47	1.93
MRF	β-red	82.18	5.15	2.27	2.70
	α-red	84.99	3.55	1.88	2.22
	α-white	86.98	3.52	1.88	2.21
	total mean	84.72	4.07	2.01	2.37

When evaluating the average size of muscle fibre types, it is possible to state that in all the studied muscles, in both the genotypic groups (AA and AB), the α-white fibres were dominant. We detected the highest values of muscle fibre thickness in MRF for both the

genotypes (90.58 µm vs. 86.98 µm respectively). The values were higher in all the muscles than the total average size of muscle fibres. We detected the lowest values of muscle fibres with β-red fibres again in all the muscles of both genotypic groups. Their average thickness was at its lowest in MTB (71.36 µm, resp. 66.40 µm). The intermediary α-red fibres had mean values, and their size more or less corresponded to the total average size of muscle fibres (Table 3 and 4).

The analysis of interstitial tissue and muscular fibres

When studying the surface proportion of the individual fibres and interstitial tissues in percentages we performed an additional analysis of muscle structure. It was again demonstrated that the surface proportion of α-white fibres is the highest in all muscle groups in both genotypes *MYF 4*. The results show that the surface proportion of those fibres in contrast to their size had the lowest values in MRF in both genotypic groups AA and AB (40% and 46.60%, respectively). The results suggest that β-red and α-red fibres represent proportionally a grater percentage than α-white, even though they are the thicken ones in terms of diameter (Table 5 and 6).

Table 5
Percentage content of interstitial tissues and muscular fibres (%) – homozygotes *MYF 4-AA*
Anteil Interstitialgewebe und Muskelfasern in % bei MYF 4-AA

	Trait	Mean	Dispersion	Standard deviation	Variation, %
MTB	β-red	25.97	4.78	2.19	8.42
	α-red	14.97	3.85	1.96	13.10
	α-white	55.66	8.87	2.98	5.26
	% interstitial tissue	2.40	0.07	0.25	10.62
MLT	β-red	17.68	2.07	1.44	8.14
	α-red	27.00	2.53	1.59	5.89
	α-white	51.80	4.46	2.11	4.08
	% interstitial tissue	3.51	0.08	0.28	7.91
MRF	β-red	24.68	2.17	3.41	13.81
	α-red	31.52	45.78	6.77	21.47
	α-white	40.00	34.23	5.85	14.63
	% interstitial tissue	3.80	0.24	0.48	12.76

Table 6
Percentage content of interstitial tissues and muscular fibres (%) – homozygotes *MYF 4-AB*
Anteil Interstitialgewebe und Muskelfasern in % bei MYF 4-AB

	Trait	Mean	Dispersion	Standard deviation	Variation, %
MTB	β-red	28.73	2.17	1.47	5.21
	α-red	13.26	0.96	0.98	7.40
	α-white	54.40	2.44	1.56	2.87
	% interstitial tissue	3.61	0.14	0.37	10.36
MLT	β-red	25.06	2.82	1.68	2.28
	α-red	15.44	3.08	1.76	11.37
	α-white	55.93	1.12	1.06	1.89
	% interstitial tissue	3.58	0.08	0.29	8.04
MRF	β-red	27.63	5.21	6.70	8.26
	α-red	21.87	5.11	2.26	10.33
	α-white	46.60	5.34	2.31	4.96
	% interstitial tissue	3.90	0.14	0.38	9.62

The results indicate that the average thickness of muscular fibres of fat cells of homozygotes *MYF 4*-AA exceeds the average thickness of muscular fibres of heterozygotes *MYF 4*-AB in all three muscles. On the basis of genetic analysis, it is necessary to consider other factors while introducing strict control in the animal population. A study performed out by FIEDLER *et al.* (2001) documents that pigs which were homozygous had a more significant ratio of carcinomatous hyperthermy in comparison to heterozygotes. This is also shown in the case of meat quality. ĽAHUČKÝ *et al.* (2000) stated that individuals with genetic and metabolic pathological defects should not be used for final hybrid production. OSTROWSKI and BLICHARSKI (1999) investigated histochemical samples of the skeletal muscles from 237 pigs (18 nn, 170 Nn and 49 NN) and did not find any statistically significant difference between the percentage of muscle content, but they demonstrated significant differences of pH in leg muscles after slaughter. NISSEN *et al.* (2004) divided pigs after weaning into the heaviest, middle-weight and light-weight according to variation of postnatal muscular fibre growth. The differences in muscular fibre growth between light-weight and middle-weight pigs were explained by the larger proportion of muscular fibres in middle-weight pigs. The differences between growth rate of middle-weight and heavy-weight pigs were explained by the higher numbers of the same large muscular fibres in the heaviest pigs. The practical value of molecular genetics is underestimated in its underutilization in the economics of rearing (WIMMERS *et al.* 2005). This fact is a result of poor knowledge of the value and utilization of selection on the basis of markers and their influence on rearing economics.

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